

ORIGINAL ARTICLE

Delayed-type allergy to cobalt—comparison of a flow cytometric lymphocyte proliferation test with patch testing

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Background: The patch test is the standard procedure for diagnosing delayed-type sensitization. If a patch test is not possible, the flow cytometric lymphocyte proliferation test (LPT), which determines the number and type of cells responding to a specific antigen in vitro, might be considered as an alternative.**Objectives:** Our aim was to establish a flow cytometric LPT for the detection of delayed-type allergic responses to cobalt, and to determine the correlation between stimulation indices (SIs) in LPT and the grade of patch test reactions. With the patch test as a diagnostic reference, we also assessed the sensitivity and specificity of the LPT.**Methods:** Fifty-four patients patch tested with the baseline series including cobalt (CoCl₂) were additionally tested with the flow cytometric LPT with CoCl₂.**Results:** There was a significant correlation between the results of both tests: $r_s = 0.43$; $P = .001$. The LPT with CoCl₂ showed a sensitivity of 52.6% and a specificity of 85.7%. Corresponding to the low sensitivity of the LPT, high likelihood ratios for a positive patch test reaction were reached only in cases of strong lymphocyte proliferation ($SI \geq 10$).**Conclusions:** In cases of clearly increased SIs, the flow cytometric LPT with CoCl₂ gives relevant diagnostic information, and represents a valuable alternative to patch testing.**KEYWORDS**

cobalt, contact allergy, flow cytometry, lymphocyte proliferation test, lymphocyte transformation test, patch test

1 | INTRODUCTION

With an average prevalence of ~6% in European patients tested for contact allergy, contact sensitization in response to cobalt represents the second most prevalent metal allergy.^{1,2} Instead of applying suspected antigens directly on the skin in the patch test,³ the proliferation of lymphocytes isolated from blood in response to the suspected allergen can be assessed with the lymphocyte proliferation test (LPT), also known as the lymphocyte transformation test.⁴ In vitro testing circumvents the problem of inducing strong local reactions, and avoids the risk of active sensitization of the patient. The LPT, however, is time-consuming and needs specialized laboratory staff and equipment. Furthermore, in the classic LPT, proliferation of

uncharacterized lymphocytes is measured by the amount of [³H]thymidine incorporation. To avoid the risk of working with radioactivity and to allow immunophenotypic characterization of proliferating cells, we have recently developed and evaluated a flow cytometric LPT with carboxyfluorescein succinimidyl ester labelling and specific staining of T cell markers for the detection of nickel and palladium sensitization.⁵

To our knowledge, results of the LPT and patch testing with cobalt have only rarely been directly compared in the literature,⁶ although this is relevant for the determination of essential diagnostic parameters of the LPT, such as sensitivity and specificity. The goal of the present study was to establish a flow cytometric LPT to determine the response to cobalt (CoCl₂), and to evaluate the correlation

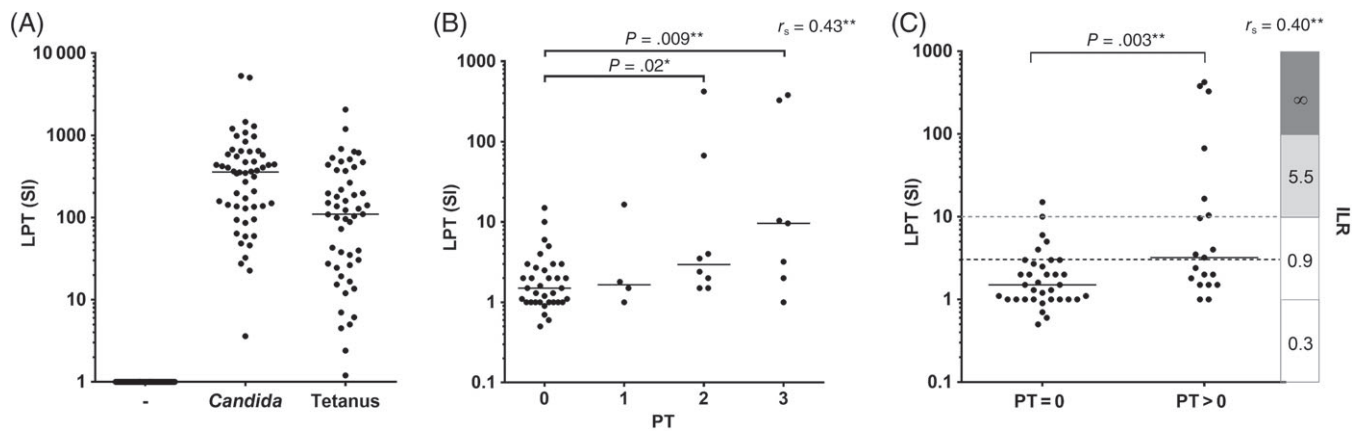


FIGURE 1 Lymphocyte proliferation test (LPT) and patch test (PT) results. **A**, Stimulation index (SI) of $CD3^+ CD4^+$ cells ($SI_{CD3^+ CD4^+}$) without antigen (-) and stimulated with *Candida albicans* or tetanus toxoid control antigens. **B**, $SI_{CD3^+ CD4^+}$ of cells exposed to $CoCl_2$, stratified by the grade of PT reaction (0-3). **C**, $SI_{CD3^+ CD4^+}$ in response to $CoCl_2$ in individuals with either negative (PT = 0) or positive (PT > 0) PT reactions. Shown are maximal SIs observed with a range of different $CoCl_2$ concentrations (0.5-29.6 $\mu\text{g/ml}$). SI cut-off values were determined by receiver operating characteristic analysis for maximal sensitivity and specificity (black dashed line) as well as 95% specificity (grey dashed line). The interval likelihood ratio (ILR) is indicated on the right of each plot. Statistical significance ($*P \leq .05$, $**P \leq .01$, $***P \leq .001$) was calculated with the Kruskal-Wallis test (B) or the Mann-Whitney test (C). Spearman's rank correlation coefficient (r_s) is indicated at the upper right corner of B and C. Generally, the group median is indicated and results are calculated with 95% confidence intervals

between results of the LPT and patch testing. Using patch testing as a diagnostic reference, we also determined the sensitivity and specificity of the flow cytometric LPT in response to cobalt.

2 | PATIENTS AND METHODS

2.1 | Patients and patch testing

Fifty-four consecutive adult patients (74% females, mean age 61 years) visiting the Allergy Unit of the Dermatology Clinic of the University Hospital Basel and eligible for patch testing with the European baseline series were included. In addition to being patch tested, each patient gave 45 mL of whole blood for the LPT with cobalt ($CoCl_2$). All patients gave informed consent to participate, and the study was approved by the local ethics committee.

Patch tests were performed with the European baseline series,⁷ including $CoCl_2$ 1% pet. (Hermal-Almirall, Wallisellen, Switzerland) in IQ Chambers (Chemotechnique Diagnostics, Vellinge, Sweden) on the upper back. The application time was 2 days, and skin reactions were read and scored on day (D) 2 and between D4 and D6, according to ESCD recommendations.⁸ Patients had been instructed not to expose test areas to ultraviolet light for 4 weeks before and during patch testing, and to stop any corticosteroid treatment 10 days prior to the patch test.

2.2 | Flow cytometric LPT

The flow cytometric LPT was performed as previously described,⁵ with *Candida albicans* and toxoid from *Clostridium tetani* as positive antigens. Cell proliferation in wells without antigen provided baseline proliferation values. $CoCl_2$ concentrations were adapted in preliminary experiments, and the final concentrations used in each assay in duplicate were 0.5, 1.0, 8.7, 13.1, 19.7 and

29.6 $\mu\text{g/ml}$ cobalt(II) chloride hexahydrate (Sigma-Aldrich, Buchs, Switzerland). As the proliferative responses of $CD3^+ CD4^+$ cells were consistently higher than those of $CD3^+ CD8^+$ cells, proliferation of $CD3^+ CD4^+$ cells is described throughout this study. The stimulation index (SI) was calculated as follows: $SI = (\text{mean number of proliferated } CD3^+ CD4^+ \text{ cells with antigen}) / (\text{mean number of proliferated } CD3^+ CD4^+ \text{ cells without antigen})$. Sensitivity, specificity and cut-off values of the LPT were calculated with the patch test as a diagnostic reference by receiver operating characteristic analysis.

3 | RESULTS AND DISCUSSION

The LPT with *C. albicans* resulted in SIs ranging from 3.6 to 5285. The response range to *Clostridium tetani* was similar, namely, 1.2 to 2060 (Figure 1A). Duplicate experiments with *C. albicans* in 43 patients indicated high precision of the flow cytometric LPT, with a mean coefficient of variation of 8.0% (SD = 9.2). On evaluation of patch test and LPT responses to $CoCl_2$ at the second reading between D4 and D6, 19 patients (35%) had a positive patch test reaction to $CoCl_2$ (+, 4 patients; ++, 8 patients; +++, 7 patients), as compared with 9 patients (16%) only showing markedly increased lymphocyte proliferation ($SI \geq 10$) in the LPT (Figure 1B,C). Furthermore, the $CoCl_2$ -specific LPT showed a significant correlation with the patch test, if stratified by different grades of patch test reactions ($r_s = 0.43$, $P = .001$; Figure 1B) and if stratified by either no patch test reaction or a positive patch test reaction ($r_s = 0.40$, $P = .003$; Figure 1C). With the patch test as a diagnostic reference, the sensitivity of the LPT was 52.6% and the specificity was 85.7% (cut-off at an SI of 3.1). With a specificity of 95%, the LPT showed a sensitivity of 31.6% (cut-off at an SI of 10.0). The low sensitivity of the LPT might be attributable to false-positive results in the patch test, as skin reactions to $CoCl_2$ can be difficult to read, and it has been shown that

only 25% of positive reactions to cobalt are clinically relevant in large patch test populations.⁹ In our study, patch tests were scored by experienced physicians (A.J.B. and K.S.) according to international standards.⁸ This is supported by the relatively high proportion of clinically relevant positive patch test reactions to CoCl_2 , namely, 52.6% (10 of 19 individuals with positive reactions). It is of note that interval likelihood ratios correlated with LPT responses, and high LPT responses ($\text{SI} \geq 10$) were almost exclusively associated with positive patch test reactions.

In conclusion, our results show that the flow cytometric LPT with cobalt can serve as an in vitro alternative in cases where patch testing is not possible. However, owing to low sensitivity, the flow cytometric LPT provides meaningful results only in patients with clearly increased SIs ($\text{SI} > 10$).

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Conflict of interest

The authors declare no potential conflict of interests. This work was funded by the University of Basel and the University Hospital Basel.

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